

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0047] with the following rewritten paragraph:

[0047] The convertase inhibitor may be a serine protease inhibitor and is preferably a thiol inhibitor. The thiol inhibitor may be a peptidyl chloroalkylketone having a peptide moiety which mimics at least one convertase enzyme cleavage site. It has been found that peptidyl chloroalkylketones with peptide moieties that mimic the convertase enzyme cleavage site are specific inhibitors of the enzymatic activity. A preferred inhibitor is decanoyl-RVCR-cmk (SEQ ID NO:1) and derivatives thereof.

Please replace paragraph [0100] with the following rewritten paragraph:

[0100] Purely by way of example an injectable solution containing between 0.1 .mu.M and 10 mM of decanoyl-RVCR-cmk (SEQ ID NO:1) is suitable for application to an existing (i.e. "open") wound.

Please replace paragraph [0125] with the following rewritten paragraph:

[0125] FIG. 2 illustrates the effect of Dec-RVCR-cmk (SEQ ID NO:1) and hexaarginine in: A platelet releasates; and B platelet-free releasates as referred to in experimental results section 2 of the example. In A .box-solid. indicates active hexaarginine and .quadrature. indicates total hexaarginine whereas .circle-solid. indicates active dec-RVCR-cmk (SEQ ID NO:1) and .smallcircle. indicates total dec-KR RVCR-cmk (SEQ ID NO:2). In panel B .smallcircle. indicates hexaarginine whereas .circle-solid. indicates dec-RVCR-k (SEQ ID NO:1); cmk; and

Please replace paragraph [0126] with the following rewritten paragraph:

[0126] FIG. 3 illustrates the effect of furin inhibitors on furin activity in cell lysates and releasates for control samples (.box-solid.); dec-RVCR-cmk (SEQ ID NO:1) (); and hexaarginine (.quadrature.) in experimental results section 2 of the example.

Please replace paragraph [0142] with the following rewritten paragraph:

[0142] In comparison platelet incubation with a membrane-permeable inhibitor of furin-like proprotein convertases, dec-RVKR-cmk (SEQ ID NO:1) (decanoyl-Arg-Val-Lys-Arg-chloromethyl ketone-Bachem), drastically reduced the generation of active TGF- β in releasates as well as intracellularly in a dose-dependent fashion (panel C). (intracellular measurements were taken from hypotonic lysates). The inventors believe that the residual TGF- β present (approximately 20-30%) was activated prematurely during platelet preparation prior to the addition of the inhibitor.

2.4. Summary.

Please replace paragraph [0146] with the following rewritten paragraph:

[0146] Platelets were activated with thrombin in the absence or presence of furin inhibitors. Platelets were pre-incubated with hexaarginine, whereas dec-RVKR-cmk (SEQ ID NO:1) was added 5 min after thrombin addition because of interference with platelet activation at higher concentrations. Active and total TGF- β in releasates were determined in the PAI/L bioassay. The results are shown in panel A of FIG. 2. Active TGF- β levels in the controls were 82 pg/ml (dec-RVKR-cmk (SEQ ID NO:1) data) and 61 pg/ml (hexaarginine data), total TGF- β levels were 33.4 ng/ml (dec-RVKR-cmk (SEQ ID NO:1) data) and 39.5 ng/ml (hexaarginine data).

Please replace paragraph [0147] with the following rewritten paragraph:

[0147] In a further experiment, furin inhibitors were added to platelet-free releasates of activated platelets, and activation was allowed to continue in the absence of platelets for 30 min at 37.degree. C. The results of this experiment are shown in panel B of FIG. 2. Active TGF- β levels in the controls were 77 pg/ml (dec-RVKR-cmk (SEQ ID NO:1) data) and 104 pg/ml (hexaarginine data). Incubation on ice reduced activation in the controls to approximately 56% (data not shown). Data represent the mean values of three independent experiments assayed in triplicate.

Please replace paragraph [0148] with the following rewritten paragraph:

[0148] The results illustrate that incubation of thrombin-stimulated platelets with the membrane-permeable protease inhibitor, dec-RVKR-cmk (SEQ ID NO:1), considerably reduces the generation of active TGF- β in releasates (FIG. 2 panel A). Dec-RVKR-cmk (SEQ ID NO:1) is a specific and potent inhibitor of subtilisin/Kex2p-like proprotein convertases, with its peptide sequence being based on the substrate recognition sequence of these enzymes.

Please replace paragraph [0150] with the following rewritten paragraph:

[0150] Latent TGF- β activation appeared to be enzymatic and independent of the continuous presence of platelets, since incubation of platelet-free releasates on ice (as compared to 37.degree. C.) reduced active TGF- β levels to approximately 56%. As observed for platelet suspensions, activation in releasates was inhibited, in a dose-dependent fashion, by the furin inhibitors, dec-RVKR-cmk (SEQ ID NO:1) and hexa-L-arginine (FIG. 2B). This indicates that the furin-like enzyme involved in latent TGF- β activation is released from activated platelets.

3.2. Platelets Contain and Release Furin-Like Enzyme Activity.

Please replace paragraph [0151] with the following rewritten paragraph:

[0151] Releasates or hypotonic lysates of activated platelets were assayed using the furin substrate, pyr-RTKR-amc (SEQ ID NO:3) in the absence or presence of the furin inhibitors, hexaarginine (200 μ M) or dec-RVKR-cmk (SEQ ID NO:1) (150 μ M). Values were corrected for substrate-independent endogenous fluorescence (control without substrate) as well as for spontaneous substrate hydrolysis (buffer control). Mean values \pm S.E.M. of 2-3 separate experiments assayed in duplicate are shown.

Please replace paragraph [0152] with the following rewritten paragraph:

[0152] The presence of furin-like enzyme activity in both hypotonic lysates and releasates of human platelets was analysed using the fluorogenic furin substrate, pyr-RTKR-amc (SEQ ID NO:3). Platelet lysates contained a furin-like enzyme activity, part of which (approximately 12%) was released upon thrombin stimulation. Enzyme activity in cell lysates and releasates was inhibited by dec-RVKR-cmk (SEQ ID NO:1) and hexa-L-arginine (FIG. 3).

Please replace paragraph [0155] with the following rewritten paragraph:

[0155] In summary, the inventors found that platelets are not only major storage sites for latent TGF- β 1 but also activate part of it following degranulation. While the mechanism of activation does not require any of the well-characterized activators, TSP-1, M6P/IGF-II receptor, or plasmin, the platelet latent TGF- β complex appears to be activated via a sequence of events by a furin-like convertase released by the platelets. Following release in vivo, this enzyme appears to continue to operate, independently of the presence of platelets, in the surrounding tissue (e.g. the wound area), leading to the activation of extracellular-matrix associated latent TGF- β complex. Therefore, this novel mechanism of activation represents a target to modulate TGF- β activity in pathologic conditions involving platelet degranulation, such as wound repair, fibrosis, arteriosclerosis, and cancer. Therefore the inventors have found that inhibitors according to the invention (such as decanoyl-RVKR-cmk (SEQ ID NO:1) and hexa-arginine may be used according to the invention).